AD

US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY 40121

REPORT NO. 745

EFFECT OF THE ROUTE OF ADMINISTRATION ON THE NEUTRALIZING POTENCY OF ANTIVENINS

(Final Report)

by
Walter F. Kocholaty, Ph.D.
Thomas A. Billings, B.S.
Billy D. Ashley, M.S.
Edith B. Ledford, A.B.
and
Joyce C. Goetz, B.A.

30 June 1967

UNITED STATES ARMY
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Disposition

Destroy this report when no longer needed. Do not return it to the originator.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

AD

REPORT NO. 745

EFFECT OF THE ROUTE OF ADMINISTRATION ON THE NEUTRALIZING POTENCY OF ANTIVENINS

(Final Report)
by

Walter F. Kocholaty, Ph. D.
Thomas A. Billings, B.S.
Billy D. Ashley, M.S.
Edith B. Ledford, A. B.
and
Joyce C. Goetz, B. A.

Biochemistry Division
US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

30 June 1967

Production of Polyvalent Antivenins
Work Unit No. 149
In-House Laboratory Independent Research
Task No. 00
In-House Laboratory Independent Research
DA Project No. 3A013001A91C

USAMRL Report No. 745
DA Project No. 3A013001A91C

ABSTRACT

EFFECT OF THE ROUTE OF ADMINISTRATION ON THE NEUTRALIZING POTENCY OF ANTIVENINS

OBJECTIVE

To elucidate the causes of some discrepancies in protective action obtained with various antivenins.

RESULTS

Using a variety of venoms, it was demonstrated that the route of administration has a decisive influence in the demonstration of the protective effect of a given antivenin.

CONCLUSIONS

In testing the efficacy of an immune serum, both intravenous and intraperitoneal administration should be given consideration.

TABLE OF CONTENTS

EFFECT OF THE ROUTE OF ADMINISTRATION ON THE NEUTRALIZING POTENCY OF ANTIVENINS

INTRODUCTION

A recent paper (1) dealing with the immunization of rabbits with photooxidized venoms of Bothrops atrox asper and of Crotalus durissus durissus described two distinctly different immunogenic responses; it appeared that the γ-globulin isolated from C. durissus durissus-immunized rabbits gave a high degree of protection against this venom, while the γ-globulin isolated from B. atrox asper-immunized animals afforded no protection against the latter venom. Immunization of rabbits with B. atrox asper venom utilizing different adjuvants led to the same result.

In another paper (2) it was reported that rabbits immunized with photooxidized venom of Naja naja showed a very weak immunogenic response.

Continuing the protection studies with mice by varying the routes of injection of immunoglobulin and of venom, it became evident that protection could be obtained. Since it seemed unlikely that the γ-globulins isolated from animals injected with B. atrox asper or Naja naja venom should be unique in this respect--one strongly hemorrhagic and necrogenic, the other a predominantly neurotoxic venom--the experiments were extended to other venom immunoglobulins. The results reported here tend to show that the route of administration is of decisive importance in the demonstration of the protective effect in the antivenom-venom interaction and that it will vary with the type of venom under study.

MATERIALS AND METHODS

All γ-globulins from rabbits or from goats were isolated according to Campbell et al (3), as described previously (1). Wyeth's antivenin (Crotalidae) polyvalent of equine origin Lot No. 07901 (expiration date, 13 December 1970), and Pentex Fraction II γ-globulin from rabbit, horse, respectively, were also used in the control experiments.

White Swiss mice, weighing 20-25 g, were used in the toxicity tests. The venoms administered were of the same origin as described in previous papers (1, 2, 4). Intravenous (I. V.) injections were given

into one of the tail veins of the mice; intraperitoneal (I. P.) injections were given into the lower left quadrant of the abdomen.

Neutralization tests were conducted by incubating 100 mg amounts* of the immunoglobulins with varying LD_{50} doses of venom for 60 minutes at 37°C in a total volume of 1.0 or 1.25 ml. Volumes of 0.20 or 0.25 ml containing 25 mg of the immunoglobulin and appropriate amounts of venom were given to mice by I. V. injection.

Protection studies were carried out by I. V. or I. P. injection of the immunoglobulins followed by the I. V. or I. P. administration of varying LD_{50} doses of venom.

Survivors were counted 24 hours after the venom was administered.

RESULTS

Naja naja. In the preceding paper (2) it was reported that only one-half of the sera (Group A, including #23-25, 31, 33) obtained from ten rabbits immunized with 22 mg of photooxidized venom of Naja naja gave a weak positive precipitin test up to the sixth dilution, while the other half (Group B, including #26-30) of the sera were practically negative. Furthermore, it was shown that γ-globulins isolated from sera of Group A gave almost no protective effect when injected I. V. and the mice challenged 30 minutes later by I. P. injection of venom produced results which indicated that a test of the protective effect of the γ-globulins isolated from sera of Group B would be superfluous. However, as shown in Table 1, when both injections were administered I. V. (γ-globulin followed by various venom doses 30 minutes later), the protective effect of the rabbit-globulins from sera of Group A was evident. Surprisingly, protection was also obtained with the γ-globulin preparation from sera of Group B.**

I. V.-I. V. protection studies with rabbit immunoglobulin from sera of Group C obtained by immunization of rabbits with double the amount of photooxidized Naja naja venom could not be performed due to lack of material.

*Twenty mg amounts were used with C. durissus durissus venom.

**This would indicate that the precipitin test carried out with whole venom is of little value as a diagnostic test where the presence of a variety of antigens and antibodies will influence their mutual solubilities.

B. atrox asper. As reported previously (1), no protection against B. atrox asper venom was obtained with a γ-globulin isolated from rabbits that had been immunized with photooxidized B. atrox asper venom. When the routes of injection were varied as in the experiments described earlier, significant differences in the degree of protection were obtained. These results are summarized in Table 2.

Agkistrodon piscivorus. The γ-globulins obtained from rabbits inoculated with photooxidized venom of Agkistrodon piscivorus reported previously (4) were used. Differences in the protection level are evident depending upon the various routes of administration. The results summarized in Table 3 show again that an I. V. - I. V. administration of immunoglobulin and venom resulted in a higher LD_{50} protection rate than an I. V. - I. P. (globulin and venom) administration.

C. atrox and C. durissus durissus.* When the polyvalent antivenin (Wyeth) was tested for its neutralization and protection properties against C. atrox venom in mice using a procedure similar to that described for venom, similarly as were the venoms described above, comparable results were obtained using the different routes of administration (Table 4).

Similar conditions prevailed when Wyeth's antivenin was examined for its neutralization and protection properties against C. durissus durissus venom (Table 5). In this case, however, the protection values for the different routes of administration are reversed when compared with the values for C. atrox and correspond to the peculiar I. V. - I. P. toxicity of this venom.

Micrurus fulvius fulvius. A recent report (6) dealt with the production of an immune serum in rabbits against the venom of the North American coral snake. These experiments were extended to goats to provide a greater amount of the immune serum. Table 6 shows the results obtained using different routes of administration and of the protective effect produced by goat γ-globulin.

The results of the studies are summarized in Table 7. The "neutralization and protection capacities" were calculated according

*Neutralization and protection experiments were reported previously using C. atrox (5) and C. durissus durissus venoms with γ-globulin derived from rabbits that had been immunized with the photooxidized venoms of these reptiles; however, the amounts of γ-globulin were insufficient for a complete study.

to Reed and Muench (7). While such a calculation is uncommon, and can at best present an approximation, it is revealing inasmuch as it demonstrates the differences obtainable by varying the route of administration. Lack of rabbit γ-globulin, antivenin prevented the completion of several neutralization experiments.

DISCUSSION

It is common to express the neutralization potency of an immune serum in LD_{50} of venom neutralized per ml of serum, and is determined by incubation of the components under standard conditions of time and temperature followed by injection of the material into mice of standard weight. Depending on the route of administration, the neutralizing ability of the serum is then expressed in the appropriate LD_{50} of venom I. V. or I. P. As indicated in Table 7, this can lead to results varying as much as 100%. The question arises as to most appropriate means for testing an immune serum. The same disparity prevails in the protection tests. Actually, neither way affords a realistic approach to the effect of venom in an actual snakebite. Envenomation rarely occurs intraperitoneally, perhaps in rare cases intravenously, but usually what could be termed intramuscularly or (perhaps with small snakes) intradermally, with extremities the primary locus of envenomation. Thus, the "neutralization" tests when conducted I. V. or I. P. simply afford a convenient and reproducible route of injection, as contrasted with an intramuscular or intradermal injection that would simulate snakebite to a closer degree.

While the common denominator derived from these investigations would appear to be for some venoms (B. atrox, M. fulvius, C. atrox, C. durissus) the amount of venom neutralized by a given amount of immune serum irrespective of the route of injection, this does not hold true for other venoms (A. piscivorus, N. naja).

With the single exception of C. durissus durissus, the degree of protection via the I. V. route is consistently higher than the I. P. administration.

The variety of physical, chemical and physiologically active components contained in snake venom and their wide range in molecular weight would make for great differences in the kinetics of drug absorption and penetration to the site of action, depending upon the route of administration. It would then seem that in order to arrive at a rational appraisal of the efficacy of immune serum, both I. V. and I. P. administration should be given consideration; at least as long as one is

uncertain of the chemical nature, pharmacological activity and toxicity of the variety of components contained in the venoms.

SUMMARY

Using a variety of venoms, it was demonstrated that the route of administration has a decisive influence in the demonstration of the protective effect of a given antivenin.

It is suggested that in testing the efficacy of an immune serum, both intravenous and intraperitoneal administration should be given consideration.

LITERATURE CITED

1. Kocholaty, W., et al. Immunogenic response of the venoms of fer-de-lance (Bothrops atrox asper) and lacascabella (Crotalus durissus durissus) following photooxidative detoxification. USAMRL Report No. 743, 1967.

2. Kocholaty, W., et al. Immunization studies with Naja naja venom detoxified by photooxidation. USAMRL Report No. 744, 1967.

3. Campbell, D. H., J. S. Garvey, N. E. Cremer and D. H. Sussdorf. Methods in Immunology. New York: W. A. Benjamin, 1963.

4. Kocholaty, W. and B. D. Ashley. Detoxification of Russell's viper (Vipera russellii) and water moccasin (Agkistrodon piscivorus) venoms by photooxidation. USAMRL Report No. 629, 1965 (DDC AD No. 466949); Toxicon, 3: 187-194, 1966.

5. Kocholaty, W. Detoxification of Crotalus atrox venom by photooxidation in the presence of methylene blue. USAMRL Report No. 628, 1965 (DDC AD No. 466948); Toxicon, 3: 175-186, 1966.

6. Kocholaty, W., B. D. Ashley and T. A. Billings. An immune serum against the North American coral snake (Micrurus fulvius fulvius) venom obtained by photooxidative detoxification. USAMRL Report No. 699, 1966 (DDC AD No. 645108).

7. Reed, L. J. and H. Muench. A simple method of estimating fifty per cent end points. Amer. J. Hyg. 21: 493, 1938.

TABLE 1

PROTECTION OF MICE AGAINST NAJA NAJA VENOM BY RABBIT IMMUNOGLOBULIN

LD_{50}	Neutralization by "A"	Protection by "A"		Protection by "B"		Venom Controls
		I. V. - I. V.	I. V. - I. P.	I. V. - I. V.	I. V. - I. P.	I. V.
1	0/3	-	3/3	-	-	15/27
2	0/3	0/4	3/3	0/5	2/4	27/27
2.5	3/3	-	-	-	-	
3	3/3	0/4	3/3	0/5	4/4	
4	3/3	2/4	2/2	1/5	4/4	
5		4/4		3/4		
6				5/5		

I. V. - I. V.; I. V. - I. P.: I. V. injection of 25 mg of rabbit immune serum, followed 30 minutes later by an I. V. or I. P. injection of the LD_{50} indicated.

Numbers are deaths to 24 hours after injection over the total number of mice used.

TABLE 2

PROTECTION OF MICE AGAINST B. ATROX ASPER VENOM BY RABBIT IMMUNOGLOBULIN

LD_{50}	Neutralization* I. V.	Protection* I. V. - I. V.	Protection* I. V. - I. P.	Controls (I. V.) Venom Alone	Controls (I. V.) Venom and Normal Rabbit γ-Globulin**
1	0/4	-	0/3	3/5	3/4
2	0/4	0/5	0/3	5/5	4/4
3	0/4	1/5	3/3		
4	0/4	0/5	3/3		
5	0/4	1/5	3/3		
6	0/4	0/5	3/3		
7.5	4/4	3/5	3/3		
10	4/4	5/5	3/3		

*By immunoglobulin obtained from rabbits immunized with photooxidized venom of B. atrox asper.

**Pentex rabbit γ-globulin.

Numbers are deaths to 24 hours after injection over the total number of mice used.

TABLE 3

PROTECTION OF MICE AGAINST AGKISTRODON PISCIVORUS VENOM BY RABBIT IMMUNOGLOBULIN

LD_{50}	Neutralization* I. V.	Protection* I. V. - I. V.	Protection* I. V. - I. P.	Controls (I. V.) Venom Alone	Controls (I. V.) Venom and Normal Rabbit γ-Globulin**
1	0/3	-	-	6/8	3/4
2	0/3	0/4	0/3	8/8	4/4
3	0/3	0/4	1/3		
4	1/3	1/4	3/3		
5		3/4			
6		3/4			
7.5		4/4			

*By γ-globulin obtained from rabbits immunized with photooxidized venom of Agk. piscivorus.

**Pentex rabbit γ-globulin.

Numbers are deaths to 24 hours after injection over the total number of mice used.

TABLE 4

PROTECTION OF MICE AGAINST C. ATROX VENOM WITH WYETH'S ANTIVENIN

LD_{50}	Neutralization		Protection		Venom Only		Venom and Normal Globulin*
	I. V.	I. P.	I. V. - I. V.	I. V. - I. P.	I. V.	I. P.	I. V. - I. P.
1	-	-	-	-	0/4	4/8	4/8
2	-	-	-	-	4/4	6/8	6/8
3	0/4	-	-	-			
4	0/4	0/4	0/4	0/4			
5	0/4	2/4	0/4	3/4			
7.5	0/4	4/4	3/4	4/4			
8	4/4	4/4	4/4	4/4			
9	4/4						
10	4/4						

*Pentex horse γ-globulin.

Numbers are deaths to 24 hours after injection over total numbers of mice used.

TABLE 5

PROTECTION STUDIES WITH CROTALUS DURISSUS DURISSUS VENOM IN MICE WITH WYETH'S ANTIVENIN

	Neutralization		Protection		Venom Alone		Venom and Normal Rabbit γ-Globulin*	
LD_{50}	I.V.	I.P.	I.V.-I.V.	I.V.-I.P.	I.V.	I.P.	I.V.-I.V.	I.P.
1	-	-	-	-	12/16	11/12	3/4	2/4
2	-	-	-	-	16/16	12/12	4/4	4/4
3	0/4	0/4	0/4	0/4				
4	0/4	0/4	1/4	0/4				
5	-	0/4	3/4	0/4				
6	0/4	0/4	3/4	1/4				
8	1/4	0/4	4/4	0/4				
10	4/4	0/4	4/4	1/4				
12		0/4	4/4	5/8				
14		0/4		4/4				
16		0/4		4/4				
18		2/4						
20		7/8						
22		4/4						
24		4/4						

*Pentex, rabbit γ-globulin.

Numbers are deaths to 24 hours after injection over the total number of mice used.

Due to the high neutralization ability of this serum, only 5 mg of antivenin were used per test.

TABLE 6

PROTECTION STUDIES AGAINST MICRURUS FULVIUS FULVIUS VENOM IN MICE BY GOAT GLOBULIN*

LD_{50}	Neutralization		Protection*		Venom Control	
	I.V.	I.P.	I.V.	I.P.	I.V.	I.P.
1	-	-	-	-	4/4	2/3
2	-	-	-	-	4/4	7/8
2.5	-	-	-	1/4		
3	-	-	-	0/5		
4	0/4	0/4	0/4	0/3		
5	0/3	0/4	-	3/4		
6	4/4	1/4	1/4	-		
7	-	-	3/3	-		
7.5	-	-	-	4/4		
8	4/4	1/4	3/3			
10		2/3				
12		4/4				

*Twenty-five mg goat γ-globulin for each test.

Numbers are deaths to 24 hours after injection over the total number of mice used.

TABLE 7

"NEUTRALIZATION AND PROTECTION CAPACITIES" OF VARIOUS IMMUNOGLOBULINS USING DIFFERENT ROUTES OF INJECTION

Venom:		N. naja A	N. naja B	B. atrox asper	Agk. piscivorus	C. atrox*	C. d. durissus	M. f. fulvius**
Test: Neutralization	I.V.	2.2	3.0	6.8	4.0	7.8	7.4	5.6
" 1)	I.P.	-	-	-	-	4.0	18.2	8.8
Protection	I.V. - I.V.	4.0	4.6	6.9	5.0	6.7	4.6	6.3
"	I.V. - I.P.	0	2.0	2.5	3.5	4.5	9.6	4.4
Toxicity: LD_{50}/ 20-25 g mouse	I.V.	6		28	46	60	28	8.5
	I.P.	6		64	109	98	15	15
Protection x LD_{50}	I.V. - I.V.	24	28	193	184	402	129	54
" x LD_{50}	I.V. - I.P.	0	12	160	273	441	144	66

(for 25 mg γ-globulin)

1) Lack of γ-globulin prevented completion of these tests.

A) γ-globulin isolated from rabbit sera # 23-25, 31, 33.

B) γ-globulin isolated from rabbit sera # 26-30.

*Wyeth's antivenin.

**Goat γ-globulin.

All neutralization and protection values pertain to 25 mg of γ-globulin except C. durissus durissus where 5 mg was used.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
US Army Medical Research Laboratory
Fort Knox, Kentucky 40121

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
EFFECT OF THE ROUTE OF ADMINISTRATION ON THE NEUTRALIZING POTENCY OF ANTIVENINS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Final Report

5. AUTHOR(S) (First name, middle initial, last name)
Walter F. Kocholaty, Thomas A. Billings, Billy D. Ashley, Edith B. Ledford, and Joyce C. Goetz

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
30 June 1967	12	7

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 3A013001A91C

c. Task No. 00

d. Work Unit No. 149

9a. ORIGINATOR'S REPORT NUMBER(S)
745

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT
Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
US Army Medical Research and Development Command, Washington, D.C. 20315

13. ABSTRACT

Using a variety of venoms, it was demonstrated that the route of administration has a decisive influence in the demonstration of the protective effect of a given antivenin. (U)

It is suggested that in testing the efficacy of an immune serum, both intravenous and intraperitoneal administration should be given consideration. (U)

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED
Security Classification

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Venoms						
Immune Serums						
Immunization						
Toxoid						
Toxicology						

AG 1870-1-Army-Knox-Oct 67-90

UNCLASSIFIED

Security Classification